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## **Comparative Susceptibility of Selected Avian and Mammalian Species to a Hong Kong–Origin H5N1 High-Pathogenicity Avian Influenza Virus**

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**SUMMARY.** Seventeen avian species and two mammalian species were intranasally inoculated with the zoonotic A/chicken/Hong Kong/220/97 (chicken/HK) (H5N1) avian influenza (AI) virus in order to ascertain a relative range of susceptible hosts and the pathobiology of the resultant disease. A direct association was demonstrated between viral replication and the severity of disease, with four general gradations being observed among these species. These gradations included the following: 1) widespread dissemination with rapid and high mortality, 2) neurological disease relative to viral neurotropism, 3) asymptomatic infection or only mild transient depression associated with minor viral replication, and 4) absence of disease relative to minimal to no viral replication. This investigation not only demonstrates that the chicken/HK virus could infect multiple avian species, but also that the virulence of the chicken/HK virus varied significantly among avian species, including those species that are members of the same order.

**RESUMEN.** Susceptibilidad comparativa de especies aviarias y mamíferas seleccionadas frente al virus altamente patógeno H5N1 de influenza aviar originado en Hong Kong.

Se inocularon 17 especies aviarias y 2 especies mamíferas con el virus zoonótico de influenza aviar A/Pollo/Hong Kong/220/97 (Pollo/HK) (H5N1) por vía intranasal con el fin de verificar el rango relativo de huéspedes susceptibles y la patobiología de la enfermedad producida. Se demostró una asociación directa entre la replicación viral y la severidad de la enfermedad con cuatro estadios diferentes observados entre estas especies, los cuales incluyeron: 1) amplia diseminación con una mortalidad rápida y alta, 2) enfermedad neurológica relacionada con el neurotropismo viral, 3) infección asintomática o depresión pasajera suave asociada con una replicación viral menor, y 4) ausencia de enfermedad relacionada con una replicación viral mínima o ausente. Esta investigación no solamente demuestra que los virus Pollo/HK pueden infectar múltiples especies aviarias sino que su virulencia varía significativamente entre las especies aviarias, incluyendo aquellas especies pertenecientes al mismo orden.

**Key words:** avian influenza, avian influenza virus, avian species, immunohistochemistry, mammalian species, order Anseriformes, order Casuariiformes, order Charadriiformes, order Columbiformes, order Galliformes, order Passeriformes, order Psittaciformes, pathogenesis, pathology

**Abbreviations:** AGP = agar gel precipitin; AI = avian influenza; BHI = brain heart infusion; BSL-3Ag = biosafety level-3 agriculture; Chicken/HK = A/chicken/Hong Kong/220/97 (H5N1); DPI = days postinoculation; ELD<sub>50</sub> = mean embryo lethal dose; HE = hematoxylin and eosin; HPAI = high-pathogenicity avian influenza; IHC = immunohistochemistry; IN = intranasal; LPM = live poultry market; PDRC = Poultry Diagnostic and Research Center; SEPRL = Southeast Poultry Research Laboratory; SPF = specific pathogen free; WL = white leghorn; WR = white rock

Aquatic avian species, more specifically member species of the orders Anseriformes (ducks, geese, swans) and Charadriiformes (gulls and terns), are considered to be the primordial hosts of type A influenza viruses (31,33). Influenza viruses arising from these avian hosts intermittently transmit to other avian and even mammalian species. However, viral replication in the aberrant host is usually limited and does not typically result in overt disease (33). There have been rare exceptions in which type A influenza viruses from one host cause substantial disease in an aberrant and unrelated host. One such exception was the Hong Kong-origin H5N1 high-pathogenicity avian influenza (HPAI) virus that traversed the interclass barrier between chickens and humans and caused 18 confirmed human infections with 6 fatalities (9,10). The emergence of this virus kindled the recognition of influenza A viruses as potentially zoonotic pathogens. Fortunately, the H5N1 virus demonstrated minimal or no transmissibility among humans, and additional human cases were averted because of the poultry depopulation in Hong Kong that was implemented in December 1997.

Surveillance for H5N1 avian influenza viruses in the live poultry markets (LPMs) of Hong Kong prior to depopulation indicated that up to 20% of chickens and 2.5% of varied species of domestic and wild ducks and geese harbored H5N1 influenza viruses (29,30). However, only negative results were obtained from attempts to isolate H5N1 viruses from other gallinaceous birds, such as guinea fowl, Japanese quail, assorted pheasants, francolins, Chukar partridges, pigeons, or various caged passerine and psittacine birds, as well as from mammals associated with the LPMs, including rats (16,29). Chickens were the only species in the LPMs that were clinically affected (28,29). Furthermore, H5N1 and viruses of other subtypes carrying internal genes with similar sequences to the 1997 H5N1 HPAI virus have been isolated from other avian species including geese (H5N1), teal (H6N1), quail (H9N1), and parakeets (H9N1). Unfortunately, investigations into the epidemiology of the Hong Kong-origin H5N1 virus have yet to fully answer how this virus was maintained from the spring HPAI outbreak on Hong Kong poultry farms and its re-emergence in the LPMs late in 1997. Additionally, questions that remain to be fully answered include what traits of this HPAI virus allowed it to traverse the interclass barrier between poultry and humans and what role particular avian and mammalian species may have played in

the maintenance and spread of this zoonotic influenza virus.

The principle objectives of this investigation were to determine the susceptibility of selected avian and mammalian species to infection with the A/chicken/Hong Kong/220/97 (H5N1) AI virus and to determine intrinsic pathogenic mechanisms involved in the production of disease and death in the susceptible species. These objectives were cumulatively met by evaluating the morbidity and mortality, gross and histological lesions, and distribution of viral antigen using immunohistochemistry, and by reisolating and titrating virus from selected tissues collected at various time points after intranasal (IN) inoculation. Additional details as to the results for each avian species can be obtained from previous publications (22,23,24,25).

## MATERIALS AND METHODS

**Virus.** A stock of the A/chicken/Hong Kong/220/97 (chicken/HK) (H5N1) AI virus was produced by second passage in 10-day-old embryonating chicken eggs. Allantoic fluid from inoculated eggs was collected and diluted 1:300 in brain heart infusion (BHI) broth to obtain a final inoculum titer of  $10^{6.0}$  mean embryo lethal dose (ELD<sub>50</sub>) per bird. The chicken/HK virus was isolated by Drs. Les Sims and Kitman Dyrting (Agriculture and Fisheries Department, Hong Kong). A sham inoculum was made using sterile allantoic fluid diluted 1:300 in BHI.

**Animals.** Seven gallinaceous species were used in this investigation. These species included specific pathogen free (SPF) white leghorn (WL) chickens (*Gallus domesticus*; Southeast Poultry Research Laboratory [SEPR], Athens, GA), SPF white Plymouth Rock (WR) chickens (*G. domesticus*; SEPR), male broad-breasted white turkeys (*Meleagris gallopavo*; British United Turkeys, Lewisburg, WV), Japanese quail (*Coturnix coturnix japonicus*; Poultry Science Department, University of Georgia, Athens, GA) (J. quail), bobwhite quail (*Colinus virginianus*; Ideal Poultry, Cameron, TX) (B. quail), pearl guineafowl (*Numida meleagris*; Privett Hatchery, Portales, NM), Ringneck pheasant (*Phasianus colchicus*; Ideal Poultry), and Chukar partridges (*Alectoris chukar*; Ideal Poultry). All gallinaceous birds except turkeys were inoculated at 4 weeks of age; turkeys were inoculated at 3 weeks of age. The remaining species included 4-week-old SPF Pekin ducks (*Anas platyrhynchos*; Cornell University, Ithaca, NY), 2-week-old Embden geese (*Anser anser domesticus*; Privett Hatchery), 2-week-old emus (*Dromaius novaehollandiae*; Comer, GA), 4-week-old pigeons (*Columba livia*; Bokhari Squab Farm, Modesto, CA), young adult zebra finches (*Taeniopygia guttata*; Athens, GA) (Z. finches), and young adult budgerigars

(*Melospittacus undulatus*; Athens, GA). Additionally, wild-captured adult house finches (*Carpodacus mexicanus*) (H. finches), house sparrows (*Passer domesticus*) (H. sparrows), European starlings (*Sturnus vulgaris*), and 2-week-old laughing gulls (*Larus atricilla*) were used. The wild birds were acquired through the Southeastern Cooperative Wildlife Disease Study, the University of Georgia. The wild passerines were captured by mist netting or trapping methods in Clarke and Oconee Counties, GA, and were maintained in semiconfinement facilities (Poultry Diagnostic and Research Laboratory [PDRC], Athens, GA) for no less than a 1-week period of acclimation prior to moving into biosafety level 3 agricultural (BSL-3Ag) facilities at SEPRL. Nestling laughing gulls were hand caught by personnel of the Georgia Department of Natural Resources in McIntosh County, GA. Gulls were raised in semiconfinement facilities (PDRC) until 2 weeks of age, when they were moved into BSL-3Ag facilities at SEPRL. The two mammalian species were 4-week-old SPF Simonsen albino rats (Simonsen Laboratories, Gilroy, CA) and 4-week-old SPF New Zealand white rabbits (Myrtle's Rabbitry Incorporated, Thompson Station, TN).

Each species was housed separately in stainless steel isolation cabinets or self-contained isolation units (Mark 4, Controlled Isolation Systems, San Diego, CA), ventilated under negative pressure with HEPA-filtered air, and maintained under continuous lighting. Appropriate feed and water were provided *ad libitum*. General care was provided as required by the Institutional Animal Care and Use Committee, as outlined in the *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* (12). All experiments were performed in an USDA certified BSL-3Ag facility at SEPRL (4).

**Experimental Design.** Serum samples and oropharyngeal and cloacal/rectal swabs were collected from a representative number of animals of each species prior to inoculation in order to ensure that each group of birds and mammals were not harboring influenza A viruses and were serologically negative for AI as determined with the agar gel precipitin (AGP) test.

For each species used in this investigation, the animals were divided into a control group and virus-inoculated group. Table 1 summarizes the number of each species that served as sham-inoculated controls and that were inoculated intranasally with the chicken/HK virus and sampled. The days of sample collection also are presented in Table 1. Each control group consisted of three to four animals that were inoculated via the choana or nares (IN) with 0.05 or 0.1 ml of the sham inoculum. Portions of brain, lung, and kidney were aseptically collected from each control animal at 2 or 4 days postinoculation (DPI) and 10 or 14 DPI (Table 1). Tissue samples were collected in brain-heart-infusion (BHI) medium with antibiotics (100 µg/ml gentamicin, 100 units/ml penicillin, and 5 µg/ml

amphotericin B). All rats and rabbits were sedated with a 10:1 ketamine-xylazine cocktail (20 mg/kg ketamine, rats; 10 mg/kg ketamine, rabbits) administered via the peritoneum (rats) or aural vein (rabbits) to obtain a light to moderate level of sedation for IN inoculation.

The virus-inoculated group consisted of 4 to 15 animals (Table 1). Each animal was inoculated as the controls with 0.05 or 0.1 ml of the inoculum containing  $10^{5.8}$  to  $10^{6.2}$  ELD<sub>50</sub> of the chicken/HK virus. For the gallinaceous species, an additional group of six (turkeys) or eight birds was inoculated with the virus for pathotyping (35). All control and virus-inoculated animals and moribund birds were humanely euthanatized by the intravenous administration of sodium pentobarbital (100 mg/kg body weight).

**Histopathology and immunohistochemistry.** Tissues for histopathological evaluation were fixed by submersion in 10% neutral buffered formalin, routinely processed, and embedded in paraffin. Sections were made at 5 µm and stained with hematoxylin and eosin (HE). A duplicate section was stained with immunohistochemistry (IHC) using a mouse-derived monoclonal antibody (P13C11) specific for type A influenza virus nucleoprotein antigen (SEPRL, Athens, GA) as the primary antibody. Procedures for IHC followed those previously described (22). Fast red was used as the substrate chromagen, and slides were counterstained with hematoxylin. Demonstration of viral antigen was based on chromagen deposition in the nucleus, which was often accompanied by chromagen deposition within the cytoplasm.

**Virus reisolation and titration.** Portions of brain, lung, and kidney collected from control and virus-inoculated animals of each species were stored at -70°C until virus reisolation and titration were performed. Standard procedures were used for reisolation of virus from swabs and tissue samples (14,34). Minimal detection limits for viral titers were  $10^{1.9}$  ELD<sub>50</sub>/gm of tissue for the WL and WR chickens, turkeys, B. quail, guineafowl, pheasant, partridge, ducks, geese, emus, gulls, and rabbits;  $10^{2.2}$  ELD<sub>50</sub>/gm of tissue for the J. quail;  $10^{1.35}$  ELD<sub>50</sub>/gm of tissue for the Z. finches;  $10^{1.15}$  ELD<sub>50</sub>/gm of tissue for the H. finches;  $10^{1.09}$  ELD<sub>50</sub>/gm of tissue for the sparrows and starlings;  $10^{1.01}$  ELD<sub>50</sub>/gm of tissue for the budgerigars; and  $10^{0.92}$  ELD<sub>50</sub>/gm of tissue for the rats.

## RESULTS

**Control animals.** All swabs and tissues collected from each species prior to sham or virus inoculation were negative for influenza virus. Likewise, serum collected prior to inoculation of each species did not contain anti-influenza antibodies as determined with the AGP test.

There was no morbidity, mortality, or gross lesions observed in the sham-inoculated animals.

Table 1. Experimental design, including number of each species used in the sham-inoculated control and virus-inoculated groups, the number of virus-inoculated animals sampled for histological evaluation and virus reisolation, and the resultant morbidity and mortality obtained from each species inoculated IN with the A/chicken/Hong Kong/220/97 (H5N1) avian influenza virus.

Species	No. control (DPI) <sup>A</sup>	No. virus inoculated (VI)	Number VI sampled (DPI)	Morbidity <sup>B</sup> (DPI)	Mortality <sup>B</sup> (DPI)
WL chickens	4 (2, 14)	10	5 (1)	8/8 <sup>C</sup> (1.5–2.0)	8/8 <sup>C</sup> (1.5–2.0)
WR chickens	4 (2, 14)	14	7 (1, 2)	8/8 <sup>C</sup> (1.5–2.0)	8/8 <sup>C</sup> (1.5–2.0)
J. quail	4 (2, 14)	10	4 (1, 2)	8/8 <sup>C</sup> (1.5–2.5)	8/8 <sup>C</sup> (1.5–2.5)
B. quail	4 (2, 14)	10	5 (1, 2, 3)	8/8 <sup>C</sup> (1.5–3.5)	8/8 <sup>C</sup> (2.0–3.5)
Turkeys	4 (2, 14)	11	4 (1, 2)	6/6 <sup>C</sup> (1.5–2.5)	6/6 <sup>C</sup> (2.0–2.5)
Guineafowl	4 (2, 14)	11	7 (1, 2, 3)	8/8 <sup>C</sup> (2.0–5.0)	8/8 <sup>C</sup> (2.0–5.0)
Pheasants	4 (2, 14)	15	6 (1, 2, 3)	8/8 <sup>C</sup> (2.0–4.0)	8/8 <sup>C</sup> (2.5–4.0)
Partridges	4 (2, 14)	11	8 (1, 2, 4, 5, 6)	8/8 <sup>C</sup> (3.0–6.5)	6/8 <sup>C</sup> (4.0–6.5)
Z. finches	4 (2, 10)	9	6 (2, 3, 4)	7/7 <sup>D</sup> (3–5)	7/7 <sup>D</sup> (3–5)
Geese	4 (2, 14)	11	10 (2, 4, 7, 10, 14)	5/11 (4–10)	0/11
Emus	2 (14)	2	2 (5, 14)	1/2 (8–14)	0/2
H. finches	4 (2, 14)	11	9 (2, 4, 7, 13, 14)	7/9 <sup>D</sup> (4–13)	4/7 <sup>E</sup> (6–13)
Budgerigars	4 (4, 14)	10	7 (2, 4, 6, 9)	7/8 <sup>D</sup> (5–9)	6/8 <sup>D</sup> (5–9)
H. sparrows	4 (2, 10)	7	8 (2, 7, 10, 14)	3/7 (4–7)	0/7
Ducks	4 (2, 10)	9	8 (2, 4, 7, 10)	0/9	0/9
Gulls	4 (2, 14)	8	8 (2, 4, 7, 14)	0/8	0/8
Starlings	3 (2, 14)	4	4 (4, 14)	0/4	0/4
Pigeons	4 (2, 14)	10	10 (2, 4, 7, 10, 14)	0/10	0/10
Rats	4 (2, 14)	6	6 (2, 10, 14)	0/6	0/6
Rabbits	4 (2, 14)	6	6 (4, 7, 14)	0/6	0/6

<sup>A</sup>DPI = days postinoculation.

<sup>B</sup>Number affected or dead/total number inoculated.

<sup>C</sup>Morbidity and mortality values based on pathotype group.

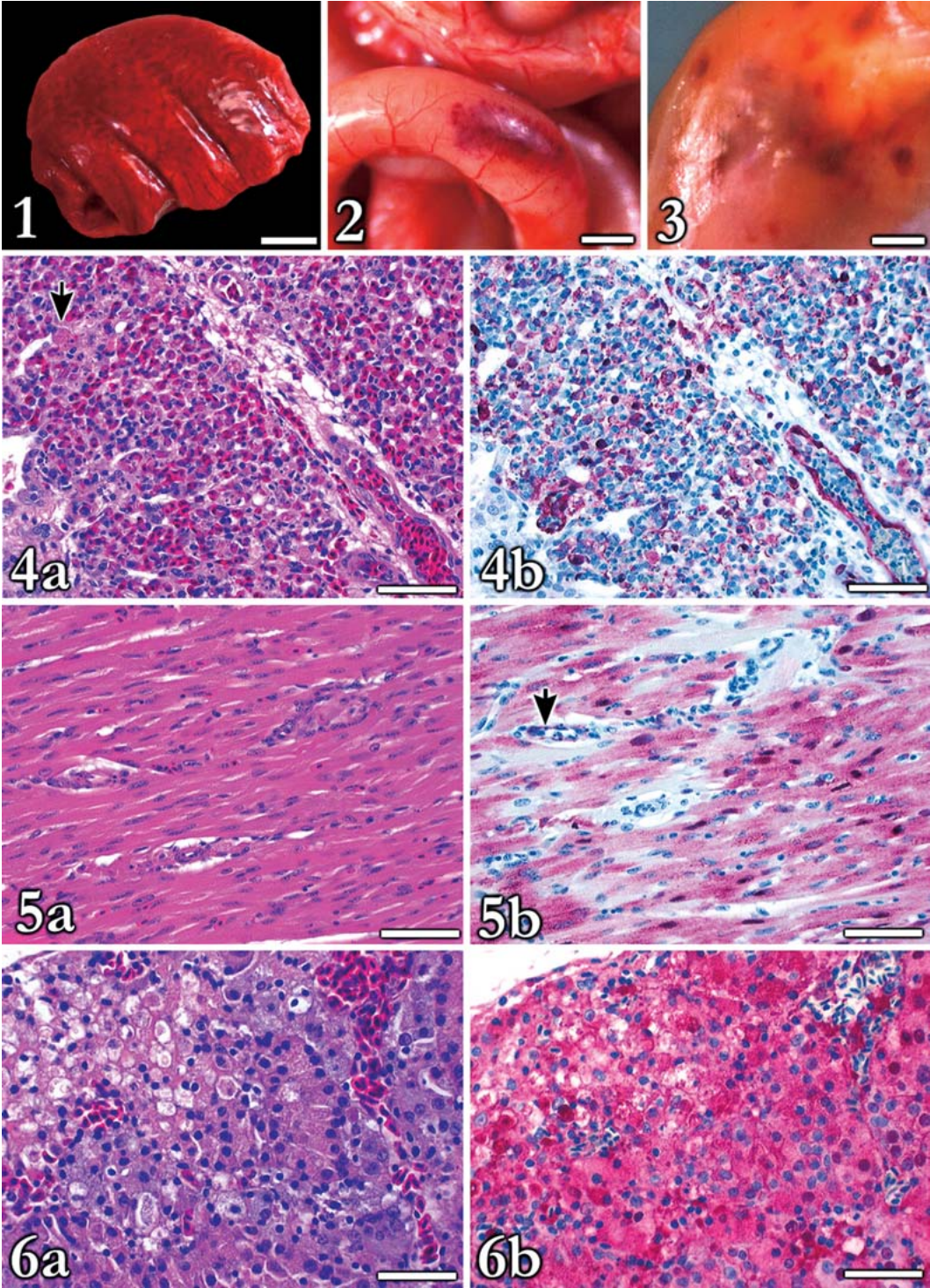
<sup>D</sup>Total morbidity and mortality counts exclude birds sampled at 2 DPI.

<sup>E</sup>Total mortality count excludes H. finches sampled at 2 and 4 DPI.

The wild passerine species had mild histological lesions related to endoparasitism. Viral antigen was not detected in tissues from the control animals. However, there was nonspecific chromagen deposition in secondary lymphoid tissues and in the submucosa of the respiratory, alimentary, and reproductive tracts in several avian species. This chromagen deposition conformed to a granular cytoplasmic distribution in individual cells and has

been previously interpreted as staining of mast cells granules (unpubl. data). Virus was not isolated from tissues collected from any of the sham-inoculated animals serving as controls.

**Virus-inoculated animals.** The chicken/HK virus demonstrated a varied ability to infect and cause disease among the avian and mammalian species used in this investigation. However, there was a direct association between the ability for the



chicken/HK virus to replicate in a species and the severity of subsequent disease observed in that particular species. These results can be categorized into to four general grades, although a degree of overlap between the grades can be discerned among some of the species investigated.

The first grade consists of the avian species found to be most susceptible to infection and included the seven gallinaceous species and the Z. finches (Table 1). For each of these species, high morbidity, characterized by severe depression with occasional neurological dysfunction in individual birds, and greater than 75% mortality within 6.5 DPI were consistently observed among these eight species (Table 1). Though gross lesions were minimal in the Z. finches, they were often present in multiple organs in the gallinaceous birds. Gross lesions in the gallinaceous birds included splenomegaly; hemorrhages in skeletal muscle, lymphoid areas of the intestinal tract, and along serosal surfaces; accumulation of serous exudates in body cavities; pulmonary edema and congestion; and mottling of the splenic and pancreatic parenchyma, especially in those birds that died within 2 to 3 days of inoculation (Figs. 1, 2, and 3). Histological lesions were observed in multiple organs from both the gallinaceous birds and the Z. finches and were exudative, hemorrhagic, necrotic, suppurative, or a combination thereof (Figs. 4a–6a). Viral antigen was demonstrated earliest in the vascular endothelium and phagocytic leukocytes (Fig. 4b), but this

was rapidly followed by the localization of viral antigen in association with the presence of histological lesions in the parenchymal cells of multiple organs. In particular, there was a predilection for the localization of viral antigen in cardiac myocytes, adrenal corticotrophic cells, pancreatic acinar epithelial cells, and neurons and glial cells of the brain (Figs. 5b, 6b). High titers of virus were obtained consistently from portions of the brain, lung, and kidney collected from individuals of each of the eight species categorized in this grade (Table 2).

The second grade of infection and disease was observed in the geese, emus, H. finches, and budgerigars. In these birds, the chicken/HK virus demonstrated a particular predilection for localization and replication in the brain, with severe neurological disease being the result of viral neurotropism (Fig. 7). In contrast with the gallinaceous birds and Z. finches, the onset of morbidity in the geese, emus, H. finches, and budgerigars was delayed (Table 1), and the mortality among these four species varied from 0% to 75% (Table 1). Gross lesions included splenomegaly and mottling of the pancreas in the emus, geese, and H. finches and splenomegaly in the budgerigars (Fig. 8). In addition, malacia was grossly evident in the brain of two geese. Histological lesions and the distribution of viral antigen were not as widespread in these four species as compared with the gallinaceous birds (Table 2). The brain regularly contained the most severe histological lesions with which viral antigen

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Fig. 1. Generalized congestion with hemorrhage and edema in the lung from a 4-week-old WL chicken that died 1.5 days after IN inoculation with the chicken/HK virus. Bar = 0.75 cm.

Fig. 2. Focal mucosal hemorrhage visible from the serosal surface of an ileal peyer's patch from a 4-week-old WL chicken that died 1.5 days after IN inoculation with the chicken/HK virus. Bar = 0.5 cm. Reprinted with permission (22).

Fig. 3. Multifocal hemorrhage in the fascial plane of the gastrocnemius muscle (*pars intermedia*) from a 4-week-old guineafowl that died 2 days after IN inoculation with the chicken/HK virus. Bar = 0.5 cm. Reprinted with permission (22).

Fig. 4. Photomicrograph of the lung from a WL chicken that died 1.5 days after IN inoculation with the chicken/HK virus. (a) Severe congestion with microthrombosis (arrow), interstitial edema, and interstitial heterophilic infiltration. HE. Bar = 50  $\mu$ m. (b) Demonstration of AI viral antigen throughout the vascular endothelium and phagocytic leukocytes within the pulmonary parenchyma and small caliber interstitial vessels. Immunohistochemical stain. Bar = 50  $\mu$ m.

Fig. 5. Photomicrograph of the heart from a 4 week old J. quail that died 2 days after IN inoculation with the chicken/HK virus. (a) Mild perivascular edema and minimal cardiac myocyte hyalinization. HE. Bar = 50  $\mu$ m. (b) Extensive intranuclear and intracytoplasmic localization of chicken/HK viral antigen in cardiac myocytes. Viral antigen also present in vascular endothelial cells (arrow). Immunohistochemical stain. Bar = 50  $\mu$ m.

Fig. 6. Photomicrograph of the adrenal glands from a Z. finch that died 5 days after IN inoculation with the chicken/HK virus. (a) Focal vacuolar degeneration to necrosis of adrenal corticotrophic and chromaffin cells. HE. Bar = 50  $\mu$ m. (b) Diffuse demonstration of AI viral antigen in the adrenal gland. Immunohistochemical stain. Bar = 50  $\mu$ m.

Table 2. Summary data obtained from the IN inoculation of multiple avian and mammalian species with the A/chicken/Hong Kong/220/97 (H5N1) avian influenza virus, including the average morbidity and mortality, average distribution and severity of gross and histological lesions, average distribution of viral antigen, and viral titers reisolated from brain, lung, and kidney.

Species	Morbidity <sup>A</sup>	Mortality <sup>B</sup>	Gross lesions <sup>C</sup>	Histological lesions <sup>D</sup>	Viral antigen <sup>E</sup>	Virus reisolation <sup>F</sup>
WL chickens	+++	+++	+++	+++	+++	+++
WR chickens	+++	+++	+++	+++	+++	+++
J. quail	+++	+++	+++	+++	+++	+++
B. quail	+++	+++	+++	+++	+++	+++
Turkeys	+++	+++	+++	+++	+++	+++
Guineafowl	+++	+++	+++	+++	+++	+++
Pheasants	+++	+++	+++	+++	+++	+++
Partridges	+++	+++	++	++	++	+++
Z. finches	+++	+++	+	++	+++	+++
Geese	++	—	+	++	++	++
Emus	++	—	+	++	++	++
H. finches	+++	++	+	++	+++	++
Budgerigars	+++	++	—	++	++	++
H. sparrows	+	—	—	+	+	+/-
Ducks	—	—	—	+	+/-	+
Gulls	—	—	—	+/-	—	+/-
Starlings	—	—	—	—	—	+/-
Pigeons	—	—	—	—	—	—
Rats	—	—	—	—	—	—
Rabbits	—	—	—	—	—	—

<sup>A</sup>Morbidity: +++ = ≥75%; ++ = 50% to 74%; + = <50%; — = none.  
<sup>B</sup>Mortality: +++ = ≥75%; ++ = 50% to 74%; + = <50%; — = none.  
<sup>C</sup>Gross lesions: +++ = lesions common and in multiple organs; ++ = lesions sporadic and in few organs; + = lesions infrequent; — = lesions not observed.  
<sup>D</sup>Histological lesions: +++ = lesions common and in multiple organs; ++ = lesions sporadic and in few organs; + = lesions infrequent; +/- = lesions rare and mild; — = lesions not observed.  
<sup>E</sup>Viral antigen: +++ = widespread; ++ = multifocal; + = infrequent; +/- = rare; — = no viral antigen.  
<sup>F</sup>Virus reisolation: +++ = high viral titers (≥10<sup>5.0</sup> ELD<sub>50</sub>/gm tissue) obtained consistently from all brain, lung, and kidney; ++ = high viral titers (≥10<sup>5.0</sup> ELD<sub>50</sub>/gm tissue) obtained primarily from brain; + = low to moderate viral titers obtained (≤10<sup>4.1</sup> ELD<sub>50</sub>/gm tissue) from lung and/or kidney, negative reisolation from brain; +/- = virus reisolated at low titers from only lung or kidney (≤10<sup>1.9</sup> ELD<sub>50</sub>/gm tissue); — = virus not reisolated from brain, lung, or kidney.

was associated (Fig. 9). Other affected organs in the geese, emus, and H. finches included the pancreas and heart, whereas in the budgerigars, lesions and viral antigen were largely confined to the brain (Figs. 10, 11). The highest titers of virus were obtained from the brain collected from individuals of these four species, whereas reisolation of virus titers from the lung and kidney tended to be lower and less consistent (Table 2).

The third grade of infection included the ducks, H. sparrows, and gulls in which the chicken/HK virus was found to be capable of only low-level replication (Table 2). No or only mild clinical disease (H. sparrows) and no mortality were affiliated with infection of these three species (Table 1). Gross lesions included only mild transient splenic enlarge-

ment and air sacculitis in few individual birds of each species. Histological lesions in the ducks and gulls were likewise mild and included only mild interstitial pneumonia and heterophilic to lymphoplasmacytic air sacculitis (Fig. 12). Viral antigen was not detected with immunohistochemistry in these affected tissues of these two species; however, low to moderate titers of virus were reisolated from the lung and/or kidney (Table 2). The reisolation of virus from the kidney was likely the result of inclusion of abdominal air sac overlying the kidney within the collected tissue. Conversely, the H. sparrows had mild multifocal lymphohistiocytic myocarditis and moderate to severe testicular degeneration to necrosis, both of which were associated with the presence of rare and widespread viral antigen, respectively

(Table 2) (Fig. 13). However, the chicken/HK virus was reisolated only at minimal detectable titers from the kidney of the H. sparrows sampled at 4 DPI.

Pigeons, starlings, rats, and rabbits compose the fourth grade of infection and disease. In these species, clinical disease was not observed, gross lesions related to viral infection were not observed, and histological lesions and related viral antigen were absent in all tissues examined. Furthermore, the chicken/HK virus was not reisolated (pigeons, rats, rabbits) or was singly reisolated at the minimal detectable titer (starling) from the kidney (Tables 1, 2).

## DISCUSSION

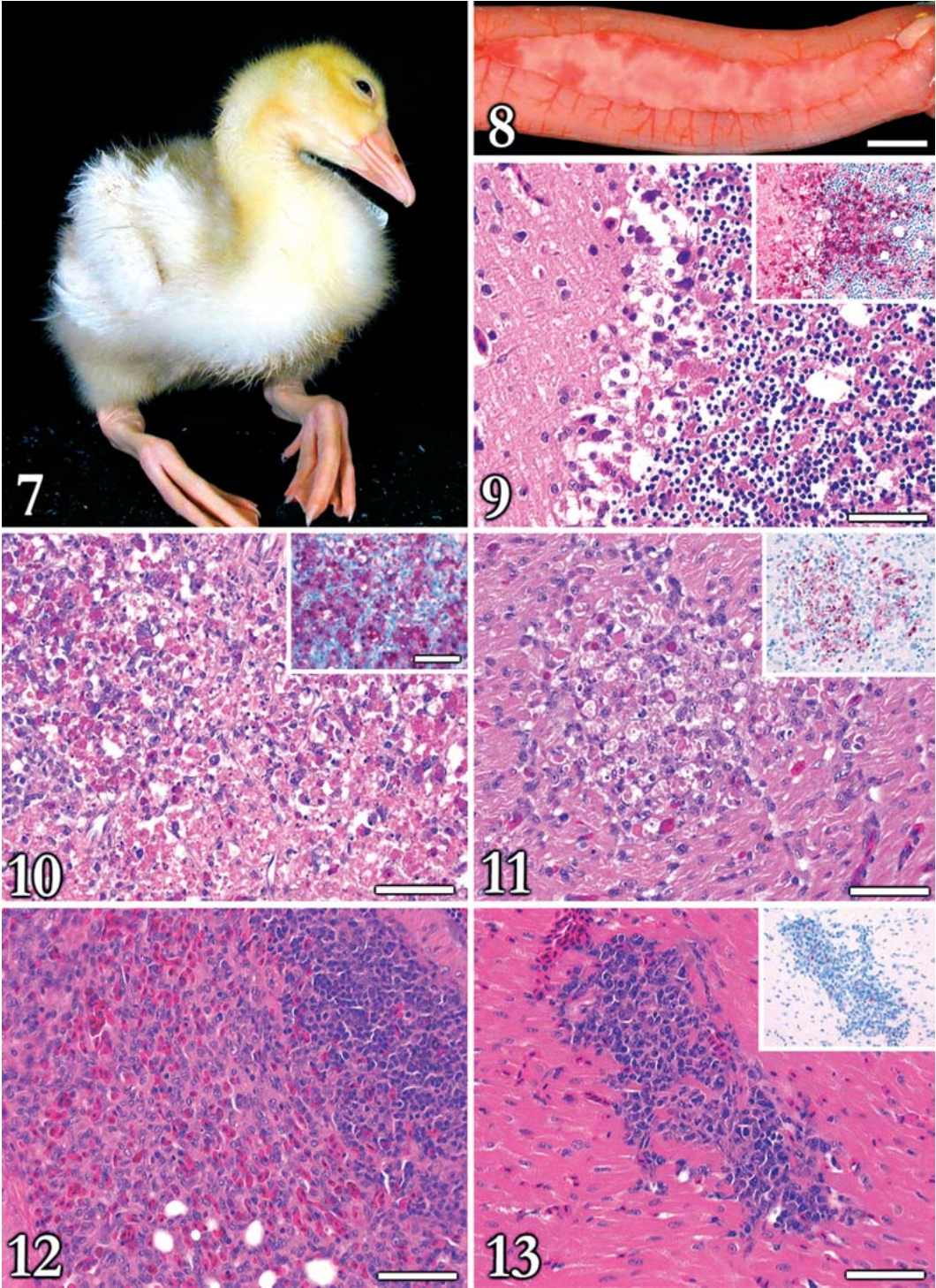
Seven gallinaceous species and Z. finches were found to be highly susceptible to infection and disease following IN inoculation with the chicken/HK virus. The disease, lesions, and distribution of viral antigen in these species are analogous to those that have been reported with other HPAI viruses in chickens and turkeys (5,17,18,19,32). Viral antigen was demonstrated in multiple tissues, confirming the pantropic nature of this HPAI virus in these avian species. However, there was a particular predilection for chicken/HK virus to localize in the vascular endothelium, heart, pancreas, brain, and adrenal glands of the gallinaceous birds and Z. finches. Based on this distribution, the underlying pathogenesis of HPAI viral infection parallels that of endotheliotropic and leukocytotropic viruses in other species, relating to a combination of factors including cardiovascular dysfunction due to endothelial activation and disruption, leukocyte activation, and systemic cytokine release; cardiopulmonary failure; and/or multiorgan failure (8,13,15,26,27,37). The rapidity of morbidity and mortality and the widespread distribution of the chicken/HK virus indicate that the chicken/HK virus is best suited for replication within these avian hosts.

Despite the disparity in morbidity and mortality between the seven gallinaceous species, Z. finches, and the geese, emus, H. finches, and budgerigars, there was an intriguing similarity in the predilection for the chicken/HK virus to localize in the brain, pancreas, and heart of these 12 species. Similarly, the few reports detailing the pathology associated with infection of geese and ratites with other HPAI viruses also have shown a predilection for HPAI viruses to localize within the brain, pancreas, and heart (6,7). Interestingly, the chicken/HK virus also was myo-cardiotropic in the H. sparrows; however, this species suffered only mild transient morbidity and no

mortality, which may have related to an absence of viral replication in the brain. In accordance with the neurological disease seen especially in the geese, emus, H. finches, and budgerigars, these results indicate that the immune-privileged nervous system is a preferred site for chicken/HK viral replication in certain avian species, especially in those birds that survive the initial stages of viremia. Furthermore, the capacity for the HPAI virus to effectively obtain entry to and replicate within the brain in a particular species is likely to be a strong determinant underlying the production of morbidity and mortality in susceptible avian species.

Contrary to these 12 species, the chicken/HK virus demonstrated only a modest ability to replicate in ducks, H. sparrows, and gulls. Furthermore, pigeons, starlings, rats, and rabbits were largely resistant to infection with the H5N1 virus. In the ducks and gulls, the chicken/HK virus was primarily pneumotropic, causing only minimal to mild lesions within respiratory tissues. These results are in accordance with previous investigations involving other poultry-origin influenza viruses in ducks and gulls (*Larus* sp.), with these viruses causing only asymptomatic infections as a result of low-level replication (1,2,3,11,36). Likewise, the available information regarding the susceptibility of pigeons to AI viruses also concurs with the results of this investigation by demonstrating that member species of the order *Columbiformes* are particularly resistant to infection with AI viruses (16,21). However, in reference to the H. sparrows and starlings, there was discrepancy between the results of this investigation and a previous report in which these two passerine species were found to be highly susceptible to the 1985 H7N7 Australian HPAI virus (20). Altogether this information would suggest that the chicken/HK virus is not necessarily exceptional in its ability to infect avian species but instead is a distinctive virus based on its ability to infect selected mammalian species without adaptation. This statement is supported both by the chicken-to-human transmission of the H5N1 virus and by previous research that demonstrates that the Hong Kong-origin H5N1 virus is highly virulent for mice as compared with other H5 HPAI viruses propagated in embryonated eggs (14).

Surveillance of the LPMs prior to the December 1997 poultry depopulation implies that only chickens, ducks, and geese may have been actively involved in the perpetuation and transmission of the zoonotic H5N1 virus (29,30). However, irrespective of the innate disparities between field and research settings, the results of this investigation suggest



that other avian species could have participated as biological vectors in the epidemiology of the H5N1 virus. Furthermore, these results give emphasis to the recognition of selected nongallinaceous species in their possible epidemiological involvement with other HPAI viruses.

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- Fig. 7. Two-week-old Embden goose with torticollis 8 days after IN inoculation with the chicken/HK virus.
- Fig. 8. Mottling of the pancreas from a 2-week-old Embden goose euthanatized 7 days after IN inoculation with the chicken/HK virus. Bar = 1 cm.
- Fig. 9. Photomicrograph of the cerebellum from an H. finch that died 13 days after IN inoculation with the chicken/HK virus. Focally extensive neuronal necrosis and vacuolation of neuropil. HE. Bar = 50  $\mu$ m. Demonstration of viral antigen in neurons and glial cells. Immunohistochemical stain.
- Fig. 10. Photomicrograph of the pancreas from a 2-week-old goose euthanatized 4 days after IN inoculation with the chicken/HK virus. Severe necrosis of the pancreatic acinar epithelium with scattered heterophils. HE stain. Bar = 50  $\mu$ m. Inset. Demonstration of chicken/HK viral antigen in pancreatic acinar epithelial cells. Immunohistochemical stain. Bar = 75  $\mu$ m.
- Fig. 11. Photomicrograph of the heart from a 2-week-old emu euthanatized 5 days after IN inoculation with the chicken/HK virus. Focal cardiac myocyte fragmentation and necrosis with mixed mononuclear inflammation. HE. Bar = 50  $\mu$ m. Inset. Demonstration of chicken/HK viral antigen in cardiac myofibers and infiltrating macrophages. Immunohistochemical stain.
- Fig. 12. Photomicrograph of the lung from a 4-week-old duck euthanatized 4 days after IN inoculation with the chicken/HK virus. Interstitial infiltration with mixed mononuclear cells and fewer heterophils adjacent to a tertiary bronchus. HE. Bar = 50  $\mu$ m.
- Fig. 13. Photomicrograph of the heart from a H. sparrow euthanatized 7 days after IN inoculation with the chicken/HK virus. Focal mononuclear infiltration with few heterophils in the myocardium. HE. Bar = 50  $\mu$ m. Inset. Demonstration of minimal viral antigen in myocytes and macrophages. Immunohistochemical stain.

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